Dietary ω 3 fatty acid alters prostaglandin synthesis, glucose transport and protein turnover in skeletal muscle of healthy and diabetic rats

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The present study was designed to determine if dietary-fat-induced alterations in the fatty acid composition of skeletal-muscle lipid alters insulin-dependent and basal muscle metabolism, including glucose and amino acid transport, prostaglandin (PG) synthesis and protein turnover. Rats were fed on high-fat semi-purified diets providing 19 % or 1 % ω 3 fatty acids in the form of fish oil, for 6 weeks. After 3 weeks, half of the rats were made diabetic by a single injection of streptozotocin (50 mg/kg body wt.). After a further 3 weeks, contralateral epitrochlearis and extensor digitorum longus (EDL) muscles from each rat were incubated *in vitro*. High levels of dietary ω 3 fatty acids decreased PGE₂ and PGF_{2 α} synthesis in EDL and epitrochlearis muscle ($P < 0.0001$). Diabetes and insulin had no effect on PG synthesis. Diet did not alter basal glucose or amino acid transport in EDL muscle from healthy or diabetic rats. Insulin increased glucose and amino acid transport ($P < 0.0001$); the increase in glucose transport by insulin was significantly greater in muscles of rats fed on high levels of ω 3 fatty acids ($P < 0.05$). Epitrochlearis from rats fed on high levels of ω 3 fatty acids showed decreased net protein degradation in the presence and absence of insulin, owing to decreased rates of protein degradation and synthesis. The data suggest that high levels of dietary ω 3 fatty acids that alter muscle membrane composition also result in alterations in glucose transport and the metabolism of muscle protein.

INTRODUCTION

It is well established in both man and animals that dietary fatty acid composition influences the composition of stored and structural lipids (Clandinin *et al.*, 1985; Berdanier, 1988; Yeagle, 1989; Murphy, 1990). Dietary-fat-induced alterations in membrane lipid composition have been shown to influence the function of membrane-associated proteins in several tissues by changing the lipid environment in which these proteins function (Field *et al.*, 1990; Clandinin *et al.*, 1985). For example, recent studies by Field *et al.* (1990) suggest that dietary fat composition within a physiological range of intake affects insulin action at the tissue level in adipocytes from healthy and diabetic rats. Insulin binding, as well as insulin-stimulated glucose transport, oxidation and lipogenesis, were significantly increased in adipocytes from rats fed on diets containing a high ratio of polyunsaturated to saturated fat. These results have important implications for management of diabetes and insulin-resistant states. Skeletal muscle is a quantitatively important insulin target tissue; thus it is of interest to establish whether effects of dietary fat could also be observed in insulin-dependent aspects of skeletal-muscle metabolism. Insulin promotes the transport of glucose into skeletal-muscle cells by specific carriers and promotes glucose oxidation as well as glycogen storage (Klip & Paquet, 1990). Insulin also promotes positive nitrogen balance in muscle by increasing the rates of amino acid uptake and protein synthesis and by suppressing the rates of protein degradation (reviewed by Kettlehut *et al.*, 1988; Sugden & Fuller, 1991).

There is little information available on the effect of different nutritional, physiological and pathological factors on the fatty acid composition of skeletal musculature, and the effects of altered membrane composition on muscle metabolism have

received little attention (Turinsky, 1986; Jackson *et al.*, 1988; Storlien *et al.*, 1991). In spite of this paucity of information, the work of several authors indirectly suggests that dietary fatty acid may influence insulin action on skeletal muscle. Storlien *et al.* (1987, 1991) reported that feeding long-chain polyunsaturated ω 3 fatty acids from fish oil diminished the development of insulin resistance in liver and muscle of rats fed on high-fat diets. A number of studies have shown that prostaglandin (PG) synthesis in several cell types is depressed by addition of long-chain ω 3 fatty acids (mainly C_{20:5 ω 3} and C_{22:6 ω 3}) in the form of fish oil to the diet (Hansen, 1986, 1989). In isolated muscle, stimulation of protein synthesis by insulin is positively correlated with PGF_{2 α} release (Reeds & Palmer, 1983; Palmer, 1990), and indomethacin (an inhibitor of PG synthesis) blocks stimulation of muscle protein synthesis by insulin both *in vitro* and *in vivo* (Reeds & Palmer, 1983; Reeds *et al.*, 1985). It also has been suggested that PGE₂ may be involved in activation of muscle protein degradation (Rodemann & Goldberg, 1982; Palmer, 1990), but the relationship between catabolic effects of PGE₂ and anabolic effects of PGF_{2 α} has not been clearly established. The present studies were therefore designed to test whether increasing dietary intake of ω 3 fatty acids alters muscle PG synthesis, glucose transport, amino acid transport and protein turnover in healthy and diabetic rats.

MATERIALS AND METHODS

Male Sprague–Dawley rats weighing 50 $\pm$ 5 g were obtained from Biosciences Animal Services, University of Alberta. Rats were randomly assigned to two diet groups and fed on semi-purified nutritionally complete diets, containing (% w/w): 27 % high-protein casein, 38 % carbohydrate (corn starch), 20 % fat,

Abbreviations used: EDL, extensor digitorum longus; MeAIB, α -methylaminoisobutyric acid; PG, prostaglandin; EMS, error mean square.

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Table 1. Fatty acid composition of the experimental diets

Fatty acid analysis of the diets was done as described in the Materials and methods section.

Fatty acid	Diet ...	Composition [% (w/w) of total fatty acids]	
		Low- ω 3	High- ω 3
C _{14:0}		1.1	2.6
C _{16:0}		12.9	10.7
C _{16:1} (ω 7)		—	3.4
C _{18:0}		17.8	10.2
C _{18:1} (ω 9)		10.4	10.0
C _{18:1} (ω 7)		0.4	1.5
C _{18:2} (ω 6)		53.8	38.7
C _{18:3} (ω 3)		1.1	0.6
C _{18:4} (ω 3)		—	2.1
C _{20:5} (ω 3)		0.1	10.9
C _{22:5} (ω 6)		—	1.1
C _{22:6} (ω 3)		0.1	5.4
Total			
Saturated		32.8	24.9
Monounsaturated		11.6	15.4
ω 6		54.2	40.7
ω 3		1.4	19.0
Polyunsaturated		55.6	59.7

1 % vitamin mixture (Association of Analytical Chemists), 5 % mineral mixture (Bernhart-Tomerelli), 0.3 % choline, 0.6 % inositol, 0.3 % methionine and 8 % non-nutritive cellulose. The complete nutrient composition of the diet has been reported in detail by Field *et al.* (1988). The diets differed only in the composition of the fat providing ω 3 fatty acids at about 1 % or 19 % of total dietary fatty acids (Table 1) in the form of fish oil (EP28; Nisshin Flour Milling Co., Tokyo, Japan). After feeding for 21 days, half the animals fed on each diet were made diabetic by a single tail-vein injection of streptozotocin (50 mg/kg body wt.) in acetate buffer (pH 4.5). The remaining animals received vehicle alone. Only animals with blood glucose levels greater than 16.6 mM were considered diabetic. Diets were fed for an additional 21 days, and the total feeding period was 6 weeks. On the last day of each experiment, rats were anaesthetized with halothane and blood samples were collected by cardiac puncture. Animals were then killed by cervical dislocation. Contralateral epitrochlearis and extensor digitorum longus (EDL) muscles were dissected immediately for incubation *in vitro* (see below).

Plasma glucose was determined with a glucose analyser (Glucose Analyzer 2; Beckman Instruments), and insulin was determined by radioimmunoassay (Insulin RIA 100 Kit; Pharmacia, Uppsala, Sweden).

Muscle protein turnover

Epitrochlearis muscles were incubated in Krebs–Ringer bicarbonate medium (NaCl 119 mM; KCl 4.8 mM; CaCl₂ 1.0 mM; NaHCO₃ 25 mM; MgSO₄ 1.25 mM; NaH₂PO₄ 1.24 mM; Hepes 2.0 mM; pH 7.4) containing additions as described below. Media were continuously gassed with O₂:CO₂ (19/1) and the temperature was maintained at 37 °C in a shaking water bath.

For determination of protein turnover, contralateral epitrochlearis muscles from each rat were preincubated for 30 min in Krebs–Ringer bicarbonate containing 5 mM-glucose, with or without addition of insulin (bovine pancreatic; Sigma Chemical Co., St. Louis, MO, U.S.A.). Studies of insulin sensitivity in

skeletal muscle are constrained by the limited availability of tissues suitable for within-animal comparisons (usually only two contralateral muscles). Therefore the tissue responsiveness (maximal stimulus) was studied, and tissues were treated with either no added insulin or 0.5 m-unit/ml (Fulks *et al.*, 1975). Muscles were transferred to fresh media of the same composition and incubated for 1 h. This medium was kept for determination of tissue PG synthesis (see below). Muscles were then incubated in the above media for an additional 3 h. Rates of net protein degradation were determined by monitoring the net release of tyrosine from protein into the incubation medium, by the methods of Fulks *et al.* (1975) using a sensitive fluorimetric assay for tyrosine (Waalkes & Udenfriend, 1957). Since tyrosine is neither synthesized nor degraded in muscle, and the intracellular pool of tyrosine does not change significantly, release of tyrosine into the medium provides an accurate measurement of net protein degradation. Rates of protein synthesis were measured with L-[2,6-³H]phenylalanine (0.05 μ Ci/ml; sp. radioactivity 56 Ci/mmol; Amersham) plus non-radioactive phenylalanine (1 mM) as described previously (Baracos & Goldberg, 1986; Strelkov *et al.*, 1989). In this approach the sum of rates of protein synthesis and of net protein degradation is equal to the rate of protein degradation, by the calculations and assumptions described by Tischler *et al.* (1981). At the end of the incubation, muscles were homogenized in 2 % trichloroacetic acid for determination of intracellular free amino acid concentrations and protein-bound radioactivity.

Glucose and amino acid transport

EDL muscles were used for determination of glucose and amino acid transport. Unlike epitrochlearis, EDL muscles from adult rats are too thick to permit adequate superfusion; therefore these muscles were divided longitudinally to form strips as described by Newsholme *et al.* (1986) before incubation. EDL muscles were mounted at their resting length *in situ* on stainless-steel supports (Baracos *et al.*, 1989). Overall, four EDL muscle strips were removed from each rat; two were used for glucose transport and two for amino acid transport. As for protein-turnover studies, the insulin-independent (no added insulin) and maximally insulin-stimulated (0.5 m-unit/ml) rates of glucose and amino acid transport were studied.

Glucose transport was estimated by using the non-metabolizable glucose analogue 3-*O*-methylglucose (Guma *et al.*, 1988, 1990). Amino acid transport was estimated with the non-metabolizable amino acid α -methylaminoisobutyric acid (MeAIB), which is taken up in skeletal muscle exclusively by system A (Guma *et al.*, 1988, 1990; Maroni *et al.*, 1986a,b). When glucose or amino acid transport was studied, [³H]inulin (0.3 μ Ci/ml; sp. radioactivity 150 mCi/g; American Radio-labelled Chemicals, St. Louis, MO, U.S.A.) plus non-radioactive inulin (1.2 μ M) were included in the incubation medium for determination of extracellular space volume (Guma *et al.*, 1988). [³H]inulin was purified before use by paper chromatography with propan-1-ol/ethanol/water (29:9:15, by vol.) as the mobile phase. Extracellular-space volumes determined for individual muscles were used in the calculations of glucose and amino acid transport.

Media for determinations of glucose transport contained 5 mM-pyruvate in place of glucose. Muscles were preincubated for 30 min in the absence or presence of added insulin, and were then transferred to fresh media of the same composition and incubated for a further 60 min. Glucose transport was then determined during a final 30 min incubation in media containing inulin and [³H]inulin as described above, and [¹⁴C]3-*O*-methylglucose (0.05 μ Ci/ml; sp. radioactivity 55 mCi/mmol; American Radio-labelled Chemicals) plus non-radioactive 3-*O*-methylglucose

(0.5 mm). Amino acid transport was determined exactly as described above for glucose transport, except that the final media contained [1-¹⁴C]MeAIB (0.05 µCi/ml; sp. radioactivity 55 mCi/mmol; American Radiolabelled Chemicals) plus non-radioactive MeAIB (0.01 mM).

After incubation, muscles were placed in tissue solubilizer (1.0 ml of Soluene; Packard), and radioactivity in muscle digests and samples of incubation media was measured by using a liquid-scintillation-counting dual-isotopic ³H/¹⁴C program. The amount of each radioisotope present in samples was determined and used to calculate rates of substrate transport corrected for extracellular space as described by Zorzano *et al.* (1985).

Prostaglandin synthesis

PG production by epitrochlearis and EDL muscles was determined by radioimmunoassay of PGE₂ and PGF_{2α} released into the incubation medium as described previously (Strelkov *et al.*, 1989). Standards were prepared in phosphate buffer, and linear standard curves were obtained over the range 2–800 pg. Rabbit anti-PGE₂ and anti-PGF_{2α} were obtained from ICN Immunobiologicals (Lisle, IL, U.S.A.) and [5,6,8,11,12,14,15-³H(n)]PGE₂ (sp. radioactivity 200 Ci/mmol) and [5,6,8,9,11,12,14,15-³H(n)]PGF_{2α} (sp. radioactivity 195 Ci/mmol) were obtained from New England Nuclear (Mississauga, Ont., Canada). The anti-PGE₂ cross-reacts 100 % with PGE₁, but by < 4 % with PGF_{2α} or its metabolites (manufacturer's data). Similarly, anti-PGF_{2α} cross-reacts 100 % with PGF_{1α}, but by < 0.1 % with PGE₂ or its metabolites. Samples of incubation media were used directly for assay without solvent extraction. Samples of the incubation media were also assayed after concentration using Sep-Pak C18 cartridges (Sigma Chemical Co.) as described by Powell (1982).

Intracellular and protein-bound amino acids were separated by h.p.l.c. using a Varian 5000 instrument with a Fluorochrom detector. Peak area integrations were obtained with a Hewlett-Packard 2625A computer. Lipids were extracted from diet by a modified Folch procedure (Folch *et al.*, 1957), and methyl esters were prepared by using 14 % (w/v) BF₃/methanol reagent and were separated by automated g.l.c. (Varian Instruments, Georgetown, Ont., Canada) as described in detail previously (Field *et al.*, 1988, 1990).

Design and statistical analysis

In total, four replicates of the experimental design were conducted, each with 7–10 rats per treatment per diet. The data presented for rats in the diabetic group was less than the number initially allocated in some experiments, since some animals died and some were excluded because of blood glucose concentrations < 16.6 mM. Glucose and amino acid transport was measured in EDL muscle in two replicates. PG synthesis was measured in three replicates, net protein degradation in four replicates, and protein synthesis in two replicates. When different replicates of the experimental design gave essentially similar results for specific variables measured, results were pooled. For some variables, e.g. Table 2, representative data are shown. The results for each treatment are presented as mean (least-square) values, and were pooled and statistically analysed by the procedure of repeated measures analysis of variance using the SAS program. Because of the method used to calculate analysis of variance and the different *n* number in various groups, it was not feasible to calculate pooled S.E.M. in a simplified way. For this reason, error mean square (EMS) values for between subjects and within subjects are given in Tables 3, 4 and 5. Pooled S.E.M. can be calculated by the following formula: $\sqrt{\text{EMS}/\text{no. of observations per treatment}}$. Data in other Tables are presented as group means $\pm$ S.E.M.

RESULTS

There were no significant effects of diet on body weight, food intake, plasma glucose and insulin concentration, or epitrochlearis muscle weight (Table 2). Plasma glucose levels were significantly higher and plasma insulin levels lower in rats injected with streptozotocin (Table 2). The diabetic animals gained significantly less body weight compared with healthy controls. However, there was no difference in the amount of food intake in control and diabetic rats. Epitrochlearis muscle weight was also significantly decreased in diabetic rats. Tyrosine and phenylalanine contents of epitrochlearis muscle protein were determined, but were not affected by diet or diabetes.

Table 2. Animal body weight, tissue weight, food intake, plasma glucose and insulin levels

The values are means $\pm$ S.E.M.: *significantly different from the respective control group (*P* < 0.001). No significant effects of diet were observed.

Treatment	Low- ω 3 diet		High- ω 3 diet	
	Control (<i>n</i> = 16)	Diabetic (<i>n</i> = 14)	Control (<i>n</i> = 16)	Diabetic (<i>n</i> = 11)
Final body wt. (g)	377 $\pm$ 7	288 $\pm$ 11*	363 $\pm$ 7	249 $\pm$ 12*
Food intake (4–6 weeks; g/day)	20 $\pm$ 1	21 $\pm$ 1	19 $\pm$ 1	17 $\pm$ 1
Plasma glucose (mM)	8.88 $\pm$ 0.17	26.6 $\pm$ 0.51*	9.16 $\pm$ 0.34	28.02 $\pm$ 1.64*
Plasma insulin (µunits/ml)	84 $\pm$ 10	26 $\pm$ 5*	117 $\pm$ 22	13 $\pm$ 2*
Epitrochlearis wt. (mg)	152 $\pm$ 3	121 $\pm$ 4*	149 $\pm$ 4	108 $\pm$ 5*

Table 3. Effect of diet on epitrochlearis-muscle PGF_{2α} and PGE₂ synthesis

Values shown are least-square means (*n* = 6). Values of EMS between and within subjects for PGF_{2α} were 2.995 and 1.497 respectively and for PGE₂ were 4.10 and 4.40 respectively. Levels of significance for various sources are indicated. Muscle prostaglandin synthesis was measured as described in the Materials and methods section. NS, not significant.

		PG synthesis (pg/h per mg of muscle)			
		Low- ω 3 diet		High- ω 3 diet	
PG	Treatment	Control	Diabetic	Control	Diabetic
PGF _{2α}	No insulin	4.71	6.01	0.85	1.31
	+ Insulin	4.21	5.01	1.36	1.73
PGE ₂	No insulin	7.83	8.86	1.49	1.97
	+ Insulin	8.16	8.86	1.88	2.41

Analysis of variance, PGE₂, PGF_{2α}

Source	<i>P</i>
Diet	0.0001
Diabetes	NS
Diet $\times$ Diabetes	NS
Insulin	NS
Diet $\times$ Insulin	NS
Diabetes $\times$ Insulin	NS
Diet $\times$ Diabetes $\times$ Insulin	NS

Table 4. Effect of diet on EDL muscle amino acid transport and glucose transport

Values shown are least-square means. Values of EMS between and within subjects were 87.67 and 35.68 respectively for amino acid transport and 443.23 and 163.48 respectively for glucose transport. Levels of significance for various sources are indicated. Muscle amino acid transport was determined as described in the Materials and methods section. NS, not significant.

	Low- ω 3 diet		High- ω 3 diet	
	Control (n = 8)	Diabetic (n = 9)	Control (n = 8)	Diabetic (n = 5)
Amino acid transport (pmol of MeAIB/ 30 min per mg of muscle)				
No insulin	29.7	48.7	30.3	53.1
+ Insulin	35.7	62.8	36.0	70.4
Stimulation (%)	20.2	28.9	18.8	32.5
Glucose transport (pmol of 3-O- methylglucose/30 min per mg of muscle)				
No insulin	87.9	106.6	80.1	94.4
+ Insulin	138.3	144.4	147.6	149.5
Stimulation (%)	57.3	35.4	84.2	58.3
Analysis of variance				
Source	Amino acid transport P		Glucose transport P	
Diet	NS		NS	
Diabetes	0.0001		NS	
Diet $\times$ Diabetes	NS		NS	
Insulin	0.0001		0.0001	
Diet $\times$ Insulin	NS		0.025	
Diabetes $\times$ Insulin	0.005		NS	
Diet $\times$ Diabetes $\times$ Insulin	NS		NS	

PGE₂ and PGF_{2 α} production

Release of PGE₂ and PGF_{2 α} by incubated epitrochlearis muscle is shown (Table 3). Dietary ω 3 fatty acids decreased synthesis of both PGE₂ and PGF_{2 α} by approx. 75 % ($P < 0.0001$). There was no effect of diabetes or insulin on muscle PG production. The results obtained with EDL muscles were essentially similar to those observed for epitrochlearis muscle, although the absolute amounts of PG synthesized were slightly lower in EDL muscle (results not shown). Because of very low levels of PG, particularly in rats fed on the high- ω 3-fatty-acid diet, radioimmunoassay of samples of the incubation media was also conducted after concentrating the samples by using Sep-Pak C18 cartridges. Values observed were similar to those illustrated (Table 3).

Glucose and amino acid transport

Basal rates of amino acid transport were approx. 30 pmol of MeAIB/30 min per mg of muscle (Table 4). In healthy rats, transport was increased by addition of insulin by about 20 %; this effect was independent of diet. Basal rates of amino acid transport were significantly elevated in diabetic rats (about 60 %), and insulin-stimulated amino acid transport was also evident (about 30 %). Diet did not alter the basal rates of muscle glucose uptake in healthy (average 84 pmol of 3-O-methylglucose/30 min per mg of muscle) or diabetic rats (average 100 pmol/30 min per mg). Insulin markedly stimulated glucose transport in all the EDL muscles; however, the increase in

Table 5. Effect of diet on epitrochlearis-muscle protein synthesis, net protein degradation and protein degradation

Values shown (in pmol of phenylalanine/3 h per mg of muscle) are least-square means. Values of EMS between and within subjects were 1125 and 417 respectively for protein synthesis, 8464 and 2125 respectively for net protein degradation and 3567 and 1641 respectively for protein degradation. Levels of significance for various sources are indicated. NS, not significant.

Treatment	Low- ω 3 diet		High- ω 3 diet	
	Control (n = 14)	Diabetic (n = 11)	Control (n = 15)	Diabetic (n = 11)
Protein synthesis				
No insulin	122	117	94	101
+ Insulin	170	166	135	155
Stimulation (%)	+39	+42	+44	+53
Protein degradation				
No insulin	532	523	449	453
+ Insulin	490	499	428	436
Inhibition (%)	8	5	5	4
Net protein degradation				
No insulin	409	418	356	356
+ Insulin	325	322	305	290
Inhibition (%)	21	23	14	19
Analysis of variance				
Source	Synthesis P	Net degradation P	Degradation P	
Diet	0.005	0.0001	0.0001	
Diabetes	NS	NS	NS	
Diet $\times$ Diabetes	NS	NS	NS	
Insulin	0.0001	0.0001	0.005	
Diet $\times$ Insulin	NS	NS	NS	
Diabetes $\times$ Insulin	NS	NS	NS	
Diet $\times$ Diabetes $\times$ Insulin	NS	NS	NS	

glucose transport by insulin was greater in muscle from healthy and diabetic rats fed on the high- ω 3-fatty-acid diet (Table 4).

Protein turnover

Initially, we wished to confirm the observation by Reeds & Palmer (1983) that indomethacin, an inhibitor of PG synthesis, inhibited the activation of muscle protein synthesis by insulin, in the epitrochlearis muscle preparation. Muscles ($n = 6$) were preincubated in Krebs-Ringer bicarbonate containing 5 mM-glucose, with or without indomethacin (1 μ M), then transferred to fresh media of the same composition, with or without insulin, and incubated for a further 2 h. The stimulation of muscle protein synthesis by insulin (+53 %) was significantly decreased (to +21 %; $P < 0.05$) in the presence of indomethacin.

The parameters of muscle protein metabolism determined (Table 5) included protein synthesis and net protein degradation. Protein degradation was calculated from these parameters. There were significant effects of diet and insulin for all parameters of muscle protein metabolism measured. However, there was no effect of the diabetic state on any of the parameters of protein metabolism studied. Insulin caused an increase in the rate of protein synthesis by about 40–50 % ($P < 0.0001$) in muscles of all animals. Muscles of all rats fed on the high- ω 3-fatty-acid diet showed decreased rates of protein synthesis (–20 %; $P < 0.05$) compared with muscles of rats fed on the low- ω 3-fatty-acid diets. Insulin suppressed the rates of net protein degradation by approx. 20 % ($P < 0.0001$). Net protein degradation rates were lowered by about 10–15 % (Table 5; $P < 0.0001$) in epitrochlearis muscles of rats fed on the high- ω 3-fatty-acid diet. The rates of protein

degradation were decreased by about 6% ($P < 0.0005$) with insulin treatment. Similarly, protein-degradation rates were inhibited by about 10% ($P < 0.0005$) in muscles of rats fed on the high- ω -3-fatty-acid diet.

DISCUSSION

Muscle fatty acid composition and PG synthesis

It is likely that decreased PG synthesis in muscles of rats fed on high levels of ω 3 fatty acids in our study reflects diet-induced changes in the fatty acid composition of muscle phospholipids. Dietary fat composition has been clearly demonstrated in a number of studies to be a major determinant of stored and structural lipids in diverse tissues (Berdanier, 1988; Field *et al.*, 1990; Murphy, 1990). Feeding animals on diets providing ω 3 fatty acids in the form of fish oil has been shown by others to alter significantly the fatty acyl composition of skeletal-muscle phospholipids (Jackson *et al.*, 1988; Storlien *et al.*, 1991). Fish oil is a polyunsaturated fat, notable for its high levels ($\sim 20\%$) of ω 3 fatty acids, particularly eicosapentanoic acid ($C_{20:5\omega3}$) and docosapentanoic acid ($C_{22:5\omega3}$). These fatty acids tend to increase in muscle phospholipids with fish-oil feeding, and are associated with a fall in levels of arachidonic acid ($C_{20:4\omega6}$) (Jackson *et al.*, 1988). A number of studies have shown that PG synthesis in several cell types is depressed by fish-oil feeding (Hansen, 1986, 1989; Galli & Simopoulos, 1989; Knapp *et al.*, 1991), and that this is related to changes in the fatty acid composition of tissue phospholipids. In particular, ω 3 fatty acids are thought: (a) to inhibit competitively the synthesis of arachidonic acid, the immediate precursor of PG (Garg *et al.*, 1988); (b) to act as alternative substrates for PG synthesis (eicosapentanoic acid), leading to the formation of the less potent triene PG (Knapp *et al.*, 1991; Galli & Simopoulos, 1989); (c) to activate PG catabolism (Grataroli *et al.*, 1988); and (d) to inhibit competitively the PG synthetase complex (eicosapentanoic acid) (Garg *et al.*, 1988). Feeding of fish-oil-based diets also tends to increase the index of unsaturation of tissue lipids, owing to its content of highly polyunsaturated fatty acids.

Although we demonstrated a diet effect on muscle PG synthesis, we were not able to demonstrate an activation of muscle PG synthesis by insulin. Reeds *et al.* (1985) reported that administration of insulin caused an increase in the PGE_2 and $PGF_{2\alpha}$ concentrations in gastrocnemius and plantaris muscles of starved young rats, and that addition of insulin to incubated rabbit muscle increased $PGF_{2\alpha}$ synthesis (Reeds & Palmer, 1983). It should be noted that the response of $PGF_{2\alpha}$ synthesis to insulin stimulation was small (Reeds & Palmer, 1983). Zorzano *et al.* (1986) reported that treatment of perfused rat hindquarter with insulin did not affect $PGF_{2\alpha}$ synthesis, but did alter the $PGE_2/PGF_{2\alpha}$ ratio through suppression of PGE_2 production. The reasons for these discrepancies are unclear, but may be related to the compartment (e.g. tissue or perfusate) in which PG levels were measured in the different experiments or other factors. Since PGE_2 is purported to have a catabolic action on skeletal muscle, and $PGF_{2\alpha}$ an anabolic action, it seems possible that one means by which these opposite effects might be exerted would be through alterations in the amounts of the two PGs formed. Our results suggest that muscle $PGE_2/PGF_{2\alpha}$ ratios are quite constant, but this question clearly requires further study.

Muscle amino acid and glucose transport

Changing the dietary ω 3 fatty acid level did not significantly affect the basal rates of glucose and amino acid transport, although basal glucose transport tended to be lower in fish-oil-fed rats. Insulin accelerated transport of both glucose and amino

acid in EDL muscle. The mechanism by which insulin mediates glucose transport in muscle is believed to involve the number of glucose transporters in the plasma membrane of the cell. This occurs due to the translocation of glucose transporters from an intracellular pool to plasma membrane (Klip & Paquet, 1990; Guma *et al.*, 1988, 1990). The mechanism by which insulin promotes amino acid transport in skeletal muscle also appears to be a direct effect of insulin at the level of the transporters, either by increasing their intrinsic activity or as a result of translocation from the intracellular pool to the plasma membrane (Guma *et al.*, 1988, 1990).

Interestingly, the activation of glucose transport by insulin was significantly greater in muscles of rats fed on the high- ω -3-fatty-acid diet. Storlien *et al.* (1987, 1991) reported that feeding long-chain polyunsaturated ω 3 fatty acids from fish oil [10% (w/w) fish oil] diminished the development of insulin resistance in liver and skeletal muscle of rats fed on high-fat diets [34% (w/w) fat]. These authors further suggested that insulin action in skeletal muscle is correlated with ω 3-fatty-acid content of membrane phospholipid. For example, insulin-stimulated 2-deoxyglucose transport correlated with the ω 3 fatty acids in muscle phospholipid (Storlien *et al.*, 1989). Our results in healthy and diabetic rats are consistent with the observations of Storlien *et al.* (1987, 1989, 1991) in insulin-resistant rats fed on high-fat diets, indicating the increased rates of insulin-stimulated glucose transport in muscle from rats fed on a high- ω -3-fatty-acid diet.

We showed an activation of insulin-dependent glucose transport in muscles of rats fed on ω 3 fatty acids, yet the insulin-dependent transport of another substrate, amino acids, was not similarly activated. These results are consistent with the work of others suggesting that the stimulation by insulin of muscle glucose and amino acid transport is mediated by different post-receptor events. For example, Sowell *et al.* (1991) recently presented evidence implicating diacylglycerol in the stimulation of muscle glucose transport by insulin. However, they did not observe any changes in the rates of amino acid transport with the increase of endogenous diacylglycerol induced by phospholipase C in isolated rat skeletal muscle. Protein kinase C has also been postulated to be involved in insulin action (Bollag *et al.*, 1986), and the observation that phorbol esters stimulate glucose transport, but not MeAIB transport, in rat skeletal muscle further supports the contention that insulin activates these transport processes by independent mechanisms (Guma *et al.*, 1988, 1990). Further work is needed to clarify the post-receptor events that lead to activation of muscle glucose transport, before the step(s) affected by dietary fat can be resolved.

Muscle protein turnover

The diet containing higher levels of ω 3 fatty acids significantly affected all of the parameters of protein turnover studied. Rats fed on the high- ω -3-fatty-acid diet showed decreased rates of protein turnover, as rates of protein synthesis and of protein degradation were both depressed. Net protein breakdown was also significantly decreased. These effects appeared to be independent of whether animals were diabetic or healthy, and were observed whether or not muscles were treated with insulin. Insulin caused a rapid rise in muscle protein synthesis, and decreased total and net protein degradation, as seen previously by others (Jefferson *et al.*, 1974; Fulks *et al.*, 1975; Goldberg *et al.*, 1980; Kettlehut *et al.*, 1988; Sugden & Fuller, 1991). Streptozotocin-induced diabetes did not influence any of the parameters of protein turnover measured in our study. There are conflicting reports in the literature about the effects of streptozotocin-induced diabetes on protein turnover measured either *in vivo* or *in vitro* (Pain *et al.*, 1983; Fagan *et al.*, 1987; Smith *et al.*, 1989; Kadowaki *et al.*, 1989). Several investigators

reported greater muscle protein breakdown in diabetic animals (Dice *et al.*, 1978; Smith *et al.*, 1989), whereas others reported a fall in protein catabolism (Millward *et al.*, 1975; Buse *et al.*, 1980) or no difference between diabetic-obese (*db/db*) and lean mice (Shargill *et al.*, 1984). The severity and/or duration of the diabetic state may be important factors in this regard. Our use of streptozotocin at 50 mg/kg body wt., as opposed to > 125 mg/kg in studies of acute severe diabetes (Pain *et al.*, 1983; Fagan *et al.*, 1987; Smith *et al.*, 1989; Kadowaki *et al.*, 1989) may account for the lack of observed effects of diabetes on protein turnover.

The mechanisms whereby dietary ω 3 fatty acids affect rates of protein synthesis and degradation are at present a matter for speculation. Since fish oil alters the fatty acid composition of cell membranes, it would be reasonable to expect that membrane-dependent processes involved in protein synthesis and degradation might be affected. For example, the suppression of protein synthesis and degradation by dietary ω 3 fatty acids was associated with dramatically lowered rates of PGE₂ and PGF_{2 α} synthesis. The fact that indomethacin significantly inhibited the stimulation of protein synthesis by insulin in isolated muscle in our study and others (Reeds & Palmer, 1983) implicates a metabolite of arachidonic acid in this effect. At the same time, PGE₂ has been suggested to activate muscle protein degradation, and indomethacin also prevents the rise in proteolysis seen in certain catabolic states (Palmer, 1990; Strelkov *et al.*, 1989). Our results are consistent with, but do not prove, a role for PG in the observed suppression of protein turnover. At the same time, dietary fatty acids could influence muscle protein metabolism by many alternative mechanisms. For example, lysosomes constitute an important membrane-associated degradative system in muscle. Effects of altered membrane composition on lysosomal function have not been characterized.

Conclusions and implications

Our results indicate that high levels of dietary ω 3 fatty acids in the form of fish oil alter muscle membrane composition, cause a marked inhibition of PGE₂ and PGF_{2 α} synthesis, suppress rates of protein synthesis and degradation, and increase responsiveness of muscle glucose transport to insulin. Fish-oil feeding may thus have implications for many aspects of muscle metabolism. Diverse processes in skeletal-muscle metabolism are thought to be PG-dependent. These include increased blood flow in active and reactive hyperaemia (Hill *et al.*, 1990), inflammation subsequent to traumatic injury (Fisher *et al.*, 1991), fusion and differentiation of myoblasts (Hausman & Berrgrun, 1987) and regulation of protein synthesis and degradation (Palmer, 1990). Rates of protein synthesis and degradation dictate whether skeletal muscle is in protein balance, or shows net protein deposition or protein loss. Both of these processes are highly regulated, and vary in a number of physiological and pathological states (Kettlehut *et al.*, 1988). The apparent ability of fish-oil feeding to suppress rates of muscle protein synthesis and degradation may have an impact on muscle growth and atrophy, and remains to be tested.

Increased glucose transport by muscles of fish-oil-fed rats may have practical implications for management of diabetes and other pathological states manifested by insulin resistance at the tissue level. Our results in this regard agree with those of Storlein *et al.* (1987, 1991), who also fed high levels of fish oil. The minimum level of dietary fish oil, as well as the necessary duration of feeding to observe this effect, remain to be clarified.

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